1: Nature. 1986 May 29-Jun 4;321(6069):522-5. Related Article

Replacing the complementarity-determining regions in a human antibody with those from a mouse.

Jones PT, Dear PH, Foote J, Neuberger MS, Winter G.

The variable domains of an antibody consist of a beta-sheet framework with hypervariable regions (or complementarity-determining regions--CDRs) whi fashion the antigen-binding site. Here we attempted to determine whether the antigen-binding site could be transplanted from one framework to another by grafting the CDRs. We substituted the CDRs from the heavy-chain variable r of mouse antibody B1-8, which binds the hapten NP-cap (4-hydroxy-3-nitrophenacetyl caproic acid; KNP-cap = 1.2 microM), for the corresponding of a human myeloma protein. We report that in combination with the B1-8 m light chain, the new antibody has acquired the hapten affinity of the B1-8 ant (KNP-cap = 1.9 microM). Such 'CDR replacement' may offer a means of constructing human monoclonal antibodies from the corresponding mouse monoclonal antibodies.

PMID: 3713831 [PubMed - indexed for MEDLINE]

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Rituxan® (Rituximab)

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WARNINGS

Fatal Infusion Reactions: Deaths within 24 hours of RITUXAN infusion have been reported. These fatal reactions followe infusion reaction complex which included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial in ventricular fibrillation or cardiogenic shock. Approximately 80% of fatal infusion reactions occurred in association with the fi infusion. (See WARNINGS and ADVERSE REACTIONS.)

Patients who develop severe infusion reactions should have RITUXAN infusion discontinued and receive medical treatmen

Tumor Lysis Syndrome (TLS): Acute renal failure requiring dialysis with instances of fatal outcome has been reported in t of TLS following treatment with RITUXAN. (See WARNINGS.)

Severe Mucocutaneous Reactions: Severe mucocutaneous reactions, some with fatal outcome, have been reported in a with RITUXAN treatment. (See WARNINGS and ADVERSE REACTIONS.)

Bac

DESCRIPTION

The RITUXAN® (Rituximab) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed agai CD20 antigen found on the surface of normal and malignant B lymphocytes. The antibody is an IgG_1 kappa immunoglobulin murine light- and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analysis) and has an approximate mole weight of 145 kD. Rituximab has a binding affinity for the CD20 antigen of approximately 8.0 nM.

The chimeric anti-CD20 antibody is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. The anti-CD20 antibody is purified by a ion exchange chromatography. The purification process includes specific viral inactivation and removal procedures. Rituxim product is manufactured from either bulk drug substance manufactured by Genentech, Inc. (US License No. 1048) or utilizin formulated bulk Rituximab supplied by IDEC Pharmaceuticals Corporation (US License No. 1235) under a shared manufacti arrangement.

RITUXAN is a sterile, clear, colorless, preservative free liquid concentrate for intravenous (IV) administration. RITUXAN is s concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single use vials. The product is formulated for IV adi in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injecti is adjusted to 6.5.

Bac

CLINICAL PHARMACOLOGY

General

Rituximab binds specifically to the antigen CD20 (human B lymphocyte restricted differentiation antigen, Bp35), a hydrophot transmembrane protein with a molecular weight of approximately 35 kD located on pre B and mature B lymphocytes.[1,2] The also expressed on > 90% of B cell non Hodgkin's lymphomas (NHL),[3] but is not found on hematopoietic stem cells, pro B ce plasma cells or other normal tissues.[4] CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation,[4] and possibly functions as a calcium ion channel.[5] CD20 is not shed from the cell surface and does not interr antibody binding.[6] Free CD20 antigen is not found in the circulation.[2]

Preclinical Pharmacology and Toxicology

Mechanism of Action: The Fab domain of Rituximab binds to the CD20 antigen on B lymphocytes, and the Fc domain recruit effector functions to mediate B-cell lysis *in vitro*. Possible mechanisms of cell lysis include complement-dependent cytotoxici and antibody-dependent cell mediated cytotoxicity (ADCC). The antibody has been shown to induce apoptosis in the DHL-4 cell lymphoma line.[8]

Normal Tissue Cross reactivity: Rituximab binding was observed on lymphoid cells in the thymus, the white pulp of the splee majority of B lymphocytes in peripheral blood and lymph nodes. Little or no binding was observed in the non lymphoid tissue examined.

Human Pharmacokinetics/Pharmacodynamics

In patients given single doses at 10, 50, 100, 250 or 500 mg/m^2 as an IV infusion, serum levels and the half-life of Rituximat proportional to dose.[9] In 14 patients given 375 mg/m^2 as an IV infusion for 4 weekly doses, the mean serum half-life was 76 (range, 31.5 to 152.6 hours) after the first infusion and 205.8 hours (range, 83.9 to 407.0 hours); after the fourth infusion.[10,1] wide range of half-lives may reflect the variable tumor burden among patients and the changes in CD20-positive (normal an malignant) B-cell populations upon repeated administrations.

RITUXAN at a dose of 375 mg/m^2 was administered as an IV infusion at weekly intervals for 4 doses to 203 patients naive t RITUXAN. The mean C_{max} following the fourth infusion was 486 µg/mL (range, 77.5 to 996.6 µg/mL). The peak and trough s levels of Rituximab were inversely correlated with baseline values for the number of circulating CD20-positive B-cells and m disease burden. Median steady-state serum levels were higher for responders compared with nonresponders; however, no was found in the rate of elimination as measured by serum half-life. Serum levels were higher in patients with International V Formulation (IWF) subtypes B, C, and D as compared with those with subtype A. Rituximab was detectable in the serum of p to 6 months after completion of treatment.

RITUXAN at a dose of 375 mg/m^2 was administered as an IV infusion at weekly intervals for 8 doses to 37 patients. The me after 8 infusions was 550 µg/mL (range, 171 to 1177 µg/mL). The mean C_{max} increased with each successive infusion throu eighth infusion (Table 1).

Table 1
Rituximab C_{max} Values

Infusion Number	Mean C_{max} µg/mL	Range µg/mL
1	242.6	16.1-581.9
2	357.5	106.8-948.6
3	381.3	110.5-731.2
4	460.0	138.0-835.8
5	475.3	156.0-929.1

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Rituxan®: Found where CD20 and hope are expressed
For relapsed or refractory low-grade or follicular, CD20+, B-cell non-Hodgkin's lymphoma (NHL).

RITUXAN TIMELINE

FIRST MONOCLONAL ANTIBODY IN THE U.S. REMAINS AN INNOVATIVE FORCE

In 1997, Rituxan became the first monoclonal antibody approved for the treatment of malignant disease in the United States, when approval was obtained for the treatment of relapsed or refractory low-grade or follicular, CD20+, B-cell non-Hodgkin's lymphoma. Since the discovery and development of Rituxan, it has become evident that its potential has yet to be fully realized, and it has become the focus of extensive clinical research throughout the world.

Since its approval, interest in Rituxan has continued to grow, with more than 200 clinical trial research protocols either completed, planned, or in progress, half of which are investigating Rituxan in patients with intermediate- and high-grade lymphoma, about 35% in low-grade NHL, and another 25% investigating its use in other indications. The number of research abstracts and clinical trial reports in the literature has risen sharply over the years, and continues to grow.

Original approval in United States based on large-scale pivotal trial

Pivotal Trial

The approval of Rituxan in the United States was based primarily on a large-scale, open-label, single-arm, multicenter study involving 166 patients with histologically confirmed low-grade or follicular NHL, which had either relapsed or was unresponsive to primary therapy. Patients received 375 mg/m² Rituxan given as a slow IV infusion once weekly for 4 doses. [5] On the basis of consultation with a panel of lymphoma experts, stringent criteria for response were established. [16]

To view the response criteria, click here.

The overall response (OR) rate was 48% (80/166) with a 6% (10/166) complete response (CR) rate and a 42% (70/166) partial response (PR) rate. The median time to onset of response was 50 days, and the median duration of response (DR) was 11.2 months. [19] All patients were treated in an outpatient setting.

To view the abstract of the pivotal trial, click here to link to the *Journal of Clinical Oncology* Web site.

Early Stage Development of Rituxan

Two-part, Phase I and II Trial

1: Hum Antibodies. 2001;10(3-4):127-42. Related Article

Engineered antibodies take center stage.

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The start of the post-genomic era provides a useful juncture for reflection on state of antibody engineering, which will be a critical technology for relating function and pathology to genomic sequence in biology and medicine. The phenomenal progress in deciphering the human genome has given significant impetus to the application of engineered antibodies in proteomics. Thus, adva in phage display antibody libraries can now help to define novel gene functio the measurement of abnormal protein expression in pathological states. Furthermore, intrabody and antibody engineering provide vehicles for the development of molecular medicines of the future. In addition to these new directions, antibody engineering has begun to show concrete success in its lo term efforts to develop targeted immunotherapies for cancer and other diseas The cornerstones of clinical development are the detailed academic clinical t that continue to push the boundaries of engineered antibodies into the real wo The field displays a healthy impatience for practical results, as research accel with concerted efforts to transfer preclinical insights into clinical trials. Grow private and governmental expenditures will lead to the rapid expansion of lif saving immunotherapeutic agents. The present review developed from our ef report on the 11th Annual International Conference on Antibody Engineering December 2000). This annual meeting is a forum for discussions on the lates advances in antibody engineering groups from around the world, and now in the broader agenda of engineering in molecular immunology. In bringing sci together to exchange ideas at this open forum, new collaborations and the thr of new discoveries are woven. For example, Professors Gerhard Wagner (Ha Medical School), Dennis Burton (Scripps Research Institute), and Peter Huds (CSIRO, Melbourne, Australia) gave exciting insights on structural immunobiology that had implications across many disciplines. The growth in antibody engineering was highlighted by the attendance of some 600 particip the meeting, doubling that of the 1999 meeting. Dramatic clinical acceptance monoclonal antibodies during the past two years has fostered this growth, wi sales in 2000 of 1.8 billion dollars and projections for 2001 of 3 billion dolla However, economic measures cannot begin to convey the medical revolution

nature

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letters to nature

Nature **363**, 446 - 448 (1993); doi:10.1038/363446a0

Naturally occurring antibodies devoid of light chains

C. HAMERS-CASTERMAN, T. ATARHOUCH, S. MUYLDERMANS, G. ROBINSON, C. HAMMERS, E. BAJYANA SONGA, N. BENDAHMAN & R. HAMMERS

RANDOM association of VL and VH repertoires contributes considerably to antibody diversity[1]. The diversity and the affinity are then increased by hypermutation in B cells located in germinal centres[2]. Except in the case of 'heavy chain' disease[3], naturally occurring heavy-chain antibodies have not been described, although antigen binding has been demonstrated for separated heavy chains[4] or cloned VH domains[5]. Here we investigate the presence of considerable amounts of IgG-like material of M_r 100K in the serum of the camel (*Camelus dromedarius*)[6]. These molecules are composed of heavy-chain dimers and are devoid of light chains, but nevertheless have an extensive antigen-binding repertoire, a finding that calls into question the role of light chains in the camel. Camel heavy-chain IgGs lack CH1, which in one IgG class might be structurally replaced by an extended hinge. Heavy-chain IgGs are a feature of all camelids. These findings open new perspectives in the engineering of antibodies.

1: Biochemistry. 2000 Feb 15;39(6):1217-22. Related Articles, L

ACS PUBLICATIONS

Camelid heavy-chain variable domains provide efficient combining sites to haptens.

Spinelli S, Frenken LG, Hermans P, Verrips T, Brown K, Tegoni M, Cambillau C.

Architecture et Fonction des Macromolecules Biologiques, CNRS, UPR-903 31 Chemiin Joseph Aiguier, 13402 Marseille Cedex 20, France.

Camelids can produce antibodies devoid of light chains and CH1 domains (Hamers-Casterman, C. et al. (1993) Nature 363, 446-448). Camelid heavy-chain variable domains (VHH) have high affinities for protein antigens and tl structures of two of these complexes have been determined (Desmyter, A. et (1996) Nature Struc. Biol. 3, 803-811; Decanniere, K. et al. (1999) Structure 361-370). However, the small size of these VHHs and their monomeric natur bring into question their capacity to bind haptens. Here, we have successfully raised llama antibodies against the hapten azo-dye Reactive Red (RR6) and determined the crystal structure of the complex between a dimer of this hapte and a VHH fragment. The surface of interaction between the VHH and the dimeric hapten is large, with an area of ca. 300 A(2); this correlates well with the low-dissociation constant of 22 nM measured for the monomer. The VHH fragment provides an efficient combining site to the RR6, using its three CDI loops. In particular, CDR1 provides a strong interaction to the hapten through two histidine residues bound to its copper atoms. VHH fragments might, therefore, prove to be valuable tools for selecting, removing, or capturing haptens. They are likely to play a role in biotechnology extending beyond protein recognition alone.

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